Annual Report of Biological Activities

C 1998 C

Newly-hatched Atlantic sturgeon

- U.S. Fish and Wildlife Service -

Northeast Fishery Center
Lamar, Pennsylvania

STUDIES PERFORMED.- In fiscal year 1998, the Northeast Fishery Center (NEFC) added culture experiments with American shad to the already demanding schedule of work involving Atlantic sturgeon, Atlantic salmon, American eels, and rainbow trout. Center biologists and support staff pooled their diverse talents once again to complete experiments which yielded useful, interesting, and thought-provoking results.

Study Number and Title:

(Previously unreported results from 1997 experiments) :

LM-97-02 Effect of two Atlantic salmon (Salmo salar) diets upon reproductive success.

LM-97-04 Effect of density on mortality of green and eyed Atlantic salmon eggs in vertical Heath-style incubator trays (Phase I - pilot study)

(Current fiscal year, 1998 studies):

LM-98-01 Effects of two broodstock diets upon rainbow trout reproductive success.

LM-98-02 Evaluation of the toxicity of various means of iodophor disinfection to Atlantic salmon (Salmo salar) eggs.

LM-98-03 Short-term marking of glass eels (Anguilla rostrata) with the fluorescent compounds, calcein and DCAF.

LM-98-04 Effect of water hardness on the uptake of oxytetracycline and calcein marks in larval Atlantic salmon caudal fin tissue.

LM-98-05 Study of larval rearing density with hatchery-produced Atlantic sturgeon (Acipenser oxyrhinchus).

LM-98-06 Comparison of growth and mortality in first-feeding Atlantic sturgeon fry when offered live Artemia sp., frozen Artemia, or a formulated diet.

LM-98-07 Study of rearing density with fingerling-size hatchery-produced Atlantic sturgeon (Acipenser oxyrhinchus).

LM-98-08 LC50 determination for three theraputic chemicals on Atlantic sturgeon (Acipenser oxyrhinchus) fingerlings.

LM-98-09 Effect of density on mortality of green and eyed Atlantic salmon eggs and size of alevins in Heath-style incubator trays at White River National Fish Hatchery (Phase II - production-scale study).

OTHER BIOLOGICAL INVESTIGATIONS PERFORMED:

LM98A Tank spawning of America shad using time-released hormone implants

LM98B Stream-side spawning of a female Atlantic sturgeon using oviduct puncture technique and transport of fertilized eggs

LM98C Pond culture of Atlantic sturgeon (feeding fry)

LM98D National Wild Fish Health Survey

LM98E Participation in the National Wild Fish Health Survey

LM98F Cooperative work on a newly found virus in Atlantic salmon

LM98G Participation in the Service National INAD Program

LM98H Participation in Maine Fish Health Advisory Board concerning ISAv issues

LM98 I Pro-active Fish Health Management at Atlantic salmon facilities

PUBLICATIONS:

DiLauro, M.N., W. Kaboord, R. Walsh, W.F. Krise, and M.A. Hendrix. 1998. Sperm-cell ultrastructure of North American sturgeons. I. The Atlantic sturgeon (Acipenser oxyrhinchus). Canadian Journal of Zoology 76:1-14.

Mohler, J.W. and J.W. Fletcher.1998. Induced spermiation in wild Atlantic sturgeons held captive up to six years. The Progressive Fish-Culturist. In progress.

TECHNICAL INFORMATION LEAFLETS:

LM-98-03. Short-term marking of American eel (Anguilla rostrata) elvers with the fluorescent compounds, calcein and DCAF.

STUDIES IN WHICH THE CENTER COOPERATED:

Improving intestinal and renal phosphate absorption in fish - *Ronaldo P. Ferraris, Department of Pharmacology and Physiology, UMD-New Jersey Medical School, Newark, NJ.*

Effects of varying dissolved oxygen levels on growth of rainbow trout - *Joseph T. Fuss, Aquatic Systems Engineering, Middlebury Center, PA*

Study Number: L-97-02

Title: Effect of two Atlantic salmon (*Salmo salar*) diets upon reproductive success

Investigators: Bill Fletcher - (NEFC) and Dale Honeyfield - U.S.G.S. Biological Resources Division (BRD)-Wellsboro,(PA)

Co-investigators: Mike Hendrix/Jerre Mohler - NEFC; Bill Krise -BRD- Wellsboro; Vic Segarich, Bob Groton - Nashua NFH (New Hampshire); Larry Lofton - North Attleboro NFH (Mass.)

Background and Justification

The Atlantic Salmon Restoration Program relies heavily upon fish cultural facilities to produce fry, parr, and smolts for restoration stocking which in turn leads to an increased demand for quality eggs. Unfortunately reproductive performance of captive broodstocks have proven inconsistent, with resultant inefficiency in culture activities. Average eye-ups between lots and years vary as much as 60%. The NEFC, other Service facilities, and Wellsboro-BRD have conducted a number of studies which have resulted in limited success in improving reproductive performance of Atlantic salmon. Broodstock nutritional requirements are poorly documented but are important to reproductive success. This study will the examine effect of diet on gamete quality and reproductive performance of Nashua NFH domestic Atlantic salmon.

Study Objectives

The objective is to determine the effect of diet on reproductive performance of Atlantic salmon broodstock fed the current standard pellet diet (ASD2-30) or a nutritionally updated extruded diet (ATS-5).

Methods

Diet.- Both diets were manufactured by Perdue Feed Inc., Catawissa, PA. The ATS-5 diet was prepared using modern extrusion technology which has been shown to improve nutrient availability. The percentage of the each ingredient in the ATS-5 was adjusted at the time of manufacturing using least cost formulation. The ATS-5 diet was formulated to : improve feed digestibility, have ingredients which may effect reproductive performance, and change the form of some nutrients vs. those used in the standard ASD2-30 diet.

Culture.- Experimental diets were fed at Nashua NFH and egg incubation was performed at North Attleboro NFH. Diets were fed for 6 months (April - September 1997). Nashua NFH set up two raceways each with 150 randomly selected 4-year-old Atlantic salmon. Individual fish lengths and weights were recorded at start of trial and at the time of spawning. Initial biomass was adjusted to ± 5 % between raceways. Adjustments in feed rate and amount was established and recorded by Nashua NFH.

Spawn.- Reproductive success was measured by evaluating 15 spawns from each diet treatment over three spawning days. Percent eye-up was determined from the first take of eggs from each female and total number of second take eggs were recorded. Each spawn was conducted as 1:1 mating. Eggs were transported to North Attleboro NFH with no initial pick of green eggs. Data were collected for the following parameters: Fin erosion indices, milt motility/viability via flow cytometry at Penn State Univ., egg shock sensitivity, and egg color. Egg samples were collected prior to fertilization for laboratory analysis of selected nutrients.

Results

Both sexes of salmon fed standard ASD2-30 diet gained more weight over the study than their counterparts (males=22% more, females=28% more). Fin condition was similar between treatments. Milt volumes were not different between treatments and egg fecundity was similar as was egg weight. Females fed the ATS-5 diet had more eggs per Kg but fish weights were lower which tended to artificially inflate egg fecundity per Kg of fish weight. Percent egg eye-up was also similar between treatments (ATS-5=39.7%; ASD2-30=42.3%). Fish fed the ATS-5 diet were less efficient at feed conversion (1.40 vs. 1.22 for ASD2-30 fish). Conversion was affected by greater moisture content of the ATS-5 diet leading to mold formation and resulting in ATS-5 treatment fish receiving more weight of feed as water. Eggs of ATS-5 fish were orange color vs. yellow in ASD2-30 fish. Future studies of this nature should be designed so experimental diets are fed for at least one year prior to spawning to allow greater time for diet effect on spermiation and oogenesis to be manifested.

Study Number: LM-97-04

Title: Effect of density on mortality of green and eyed Atlantic salmon eggs in vertically-stacked incubator trays at White River National Fish Hatchery

Investigator: Jerre W. Mohler-NEFC
Co-investigators: Ken Gillette-WRNFH

Background and Justification:

The Atlantic salmon program in Region 5 of the US Fish and Wildlife Service relies heavily upon fish cultural facilities to produce fry, parr, and smolts for restoration stocking. A large proportion of eggs produced in Region 5 salmon hatcheries are fertilized then shipped to White River National Fish Hatchery (WRNFH) for incubation due to favorable water temperatures and facilities. In 1996/97 WRNFH incubated over 9 million eggs and incubation space became scarce. Therefore it is necessary to optimize existing incubation space by determining maximum egg density per incubation tray while maintaining mortality acceptable to Region 5 managers. Normally, egg densities are maintained at 6000-8000 eyed-eggs per tray at WRNFH but the effects of density on eggs and alevins has not been studied. We propose to test the effect of density on mortality of both green and eyed eggs at WRNHF. Green egg densities to be tested are: 8,000, 10,000, and 12,000 per tray and eyed egg densities will be 6,500, 8,500, and 10,500 per tray.

Study Objectives

(1) We will compare effects of three egg densities on mortality of 150,000 green and 127,500 eyed Atlantic salmon eggs at White River NFH during the 1997/98 incubation period. (2) Based on results of this pilot-scale study, we will performed a production-scale study at WRNFH the following incubation season.

Materials and Methods

The study took place at White River NFH during the 1997/98 incubation year. After disinfection, at least 150,000 fertilized eggs were composited and gently mixed. Eggs were enumerated by displacement and placed into Heath trays at densities of 8000, 10,000, and 12,000 (± 5%) per tray. There were five replicates of each egg density. Throughout the incubation period, experimental egg trays were subject to similar treatment concerning periodic examination, shocking, and removal of dead eggs. Numbers of dead eggs in experimental trays were recorded and once production eggs reached the eyed stage, percent mortality was compared between treatments. Once eggs reached the eyed stage, they were composited, mixed, enumerated, and placed into trays at densities of 6,500, 8,500, and 10,500 per tray (± 5%). The study concluded after performing final larval counts/tray and measuring 30 individuals from each tray. Hypotheses tested were: H_o^1 : There is no difference in % mean mortality between densities. H_o^2 : There is no correlation between mortality and vertical position of heath tray for each density tested.

Results

Our results showed that at WRNFH, elevated egg incubation densities did not adversely impact % eye-up, alevin survival, size, or incidence of blue-sac and other deformities. Our highest density of 10,500 eyed eggs/tray (1.70 eggs/cm^3) was not high enough to cause deleterious effects to be observed. We found no effect of tray position on mortality, but at the 8,500 eyed-egg density, alevins were smaller with decreasing tray position. Even though our study used only five trays of each density stacked vertically, the balance of experimental trays contained eggs at normal production densities which approximated production scale at the high experimental density. Results indicate that in the event egg take exceeds normal incubation densities at WRNFH (8,000-10,000 green eggs/tray), increasing densities to 12,000 green eggs per tray reduced to 10,500 at eye-up, may not be detrimental to alevin survival and quality. This study showed that oxygen levels were depleted in lower position trays maintained at high alevin densities. Therefore, additional experimentation is necessary using a number of full incubation stacks before WRNFH can safely scale up to higher egg densities such as used in this study.

Study Number: LM-98-01

Title: Effect of two broodstock diets upon rainbow trout reproductive success

Investigators: Bill Fletcher - Northeast Fishery Center (NEFC) and Dale Honeyfield - Biological Resources Division (BRD),Wellsboro and Co-investigators - Mike Hendrix and Jerre Mohler - NEFC; Bill Krise -BRD, Wellsboro; Kari Duncan-White Sulphur Springs NFH

Background and Justification

The mission of Ennis, Erwin, and White Sulphur Springs (WSS) National Fish Hatcheries is to produce adequate numbers of disease-free, genetically distinct strains of trout eggs to support the National Fish Hatchery System as well as other federal agencies, researchers, and cooperators.
Erwin strain rainbow trout at Ennis and White Sulphur Springs NFH have shown poor eye-up (about 75%) for a number of years. Although many environmental variables are present, diet formulation can be examined without major modification of facility operations and has been demonstrated to impact egg and fry quality.

Study Objectives

The objective is to determine the effect of diet on reproductive performance of about 600 WSS NFH rainbow trout (Erwin strain) broodstock fed the current standard pellet diet (GR7-30, double vitamin pack) or a nutritionally updated extruded diet (RBT-5) in 1998.

Methods

Diet.- The experimental RBT-5 diet wasprepared using modern extrusion technology which has been shown to improve nutrient availability including addition of ingredients which may effect reproductive performance. The mineral supplements for the RBT-5 diet, was provided in an organic matrix, metal - proteinate form, which are biologically available at an increased level, compared to formulations used in GR 7-30. The RBT-5 diet contained a vitamin premix at 2.5 times more than listed requirements including vitamin C in a protected form at eight times the NRC recommended level of 50 mg/kg. Perdue Feed Inc. produced the diets. **Culture.-** The study commenced at WSS NFH in Feb. 1998, to provide a five month minimum diet period. Four raceways containing a total of 4,000 Erwin strain rainbow trout were used in the study. A total of 600 were Floy-tagged and measured at start and just prior to spawning. Two raceways each were fed either RBT-5 diet or GR7-30 diet. **Spawn.** - A total of 120 spawns were evaluated, 60 from each diet treatment. Each spawn was enumerated and incubated in partitioned Heath trays. Egg samples each group were collected prior to fertilization and frozen for nutrient analysis.

Results

The study is on-going

Study Number: LM-98-02

Title: Evaluation of the toxicity of various means of iodophor disinfection to Atlantic salmon (Salmo salar) eggs.

Investigators: Wade Jodun and Michael Millard; Northeast Fishery Center - Lamar, PA (NEFC)

Background / Justification

The eggs of Atlantic salmon and other salmonids have routinely been administered prophylactic treatments with iodine compounds to prevent transmission of viral and bacterial pathogens. In 1995, the USFWS protocol called for all salmonid eggs shipped or received at Service facilities to be water-hardened in 50 ppm active iodine for 30 minutes. Additionally, any eggs being received at Service facilities must undergo an additional 10 minute at 100 ppm active iodine. Thus, salmonid eggs could be subjected to two iodophor treatments prior to incubation. Previous studies evaluating impacts of water hardening Atlantic salmon eggs in iodophor have yielded inconclusive results, but suggest a trend towards lower egg survival with increasing time of exposure to iodophor during water-hardening. Additionally, previous studies used no controls due to disks associated with disease transmission.

Study Objectives

Primary questions to be answered by this study were: (1) Does egg disinfection with iodophor - either during or after the water hardening process result in higher mortalities? (2) Does water hardening in various concentrations of iodophor for varying lengths of time adversely impact survival of eggs when compared to water hardening eggs for one hour in plain water prior to a 10 minute exposure to 100 ppm iodophor, (3) Is egg mortality affected by an interaction between concentration and exposure time during iodophor treatments? (4) Does a second exposure to iodine adversely impact egg survival?

Materials and Methods

Eggs were taken from three female Connecticut River Domestic Atlantic salmon at NEFC and pooled to minimize variability. Milt was collected from domestic males, placed in separate containers, and held on ice. Sperm motility was assessed for each sample and samples with poor motility were discarded. Equal aliquots of milt were pooled prior to fertilization. Stock solutions of buffered iodophor at 50, 100, and 150 ppm and untreated controls were prepared to water-harden eggs for 30, 60, and 90 minutes followed by 10 minutes exposure at 100 ppm iodophor after a 4-hour time lapse. A parallel set of treatments was performed except that after a 4-hour time lapse, the 10 minute treatment contained no iodophor. Treatments described above were run in triplicate resulting in a total of 72 total challenges. All lots including controls were subjected to similar handling procedures.

Results

Statistical analysis demonstrated that time of exposure had the greatest impact on egg survival. Across all treatments, including the controls, a significant (26.2 %) decline in survival was found between 30 and 60 minutes, strongly suggesting that losses may be attributed to an increased sensitivity to handling of eggs during that post-fertilization time period. Interaction between iodophor concentration and exposure time was most evident at the high (150 ppm) iodophor concentration with significantly greater egg mortality with each increase in exposure time. Only at the 30-minute exposure did control eggs display significantly greater survival (88.3%) than iodophor treated eggs (79.7 %). Our study demonstrated that mortality of Atlantic salmon eggs was not excessive when treatments ranged from 50 - 150 ppm for 30 minutes, but longer exposure times may require concentrations of 100 ppm or less. We also found that no excessive mortality resulted from a subsequent 10 minute 100 ppm disinfection five hours following the initial treatment.

Study Number: LM-98-03

Title: Short-term marking of glass eels (Anguilla rostrata) with the fluorescent compounds, calcein and DCAF.

Investigators: Jerre W. Mohler and John W. Fletcher - Northeast Fishery Center-Lamar, PA

Background and Justification

American eels are a catadromous species whose larval form, known as leptocephali, are pelagic. During this life stage, they are transported by Atlantic Ocean currents and metamorphose to a "glass eel" stage which lacks external pigmentation. Glass eels migrate into fresh water river systems on the eastern seaboard of North America and the Gulf of Mexico. As they reach fresh water, they begin to acquire pigmentation and are known as "elvers". Harvest of these elvers as they migrate from the ocean into fresh water has increased substantially due to Asian market demands, and has created fisheries management and law enforcement problems. In 1997, the Northeast Fishery Center- Lamar, PA (NEFC) began experimenting with inducing a non-lethal mark on elvers using calcein, a fluorescent compound which readily dissolves in water and can be applied as an immersion bath. We found that the gall bladder took up the calcein mark which can be detected under ultra-violet light (UV) without magnification. As a result, this study was performed to develop a reliable technique for immersion of elvers in calcein solutions to induce a mark which cannot be seen without the aid of an ultra-violet light source. Osmotic shock methods were explored along with a variety of marking solution concentrations. Additionally, marked elvers were mixed with unmarked elvers to determine discrimination between groups using various UV light sources.

Materials and Methods

American eel elvers were captured in the Matamesquite National Wildlife Refuge, North Carolina and shipped to the NEFC. Chemical immersion tests were performed by placing elvers into 6½-L clear acrylic fish egg incubation jars along with 2 L of ambient temperature hatchery water and subjected them to a 5% non-iodized salt solution for 3.5 minutes. Salt solutions were then decanted and replaced with 1 L of various concentrations of calcein solutions: After 19 h, solutions were decanted from test jars ard replaced with fresh hatchery water of like temperature. One set of treatments was tempered to ambient water temperature (6°C) and the remaining three jars remained at room temperature. Twenty elvers were removed from each jar daily for 7 d and scored for mark retention. A variety of UV detection devices and photographic techniques were tested to capture an accurate representation of the calcein mark.

Results

We found that elvers are most efficiently marked using a concentrated calcein solution of at least 1.0% for 3.5 minutes after a 5% non-iodized salt bath for 3.5 minutes. Results of immersion trials showed that the gall bladder was the structure most prominently marked. Of the various detection devices tested, fluorescent marks were most easily identified using a commercially-available ultra-violet trans-illuminator table. At 7 d post-immersion, over 50% of marked elvers were easily discerned from unmarked when combined in a glass tray on the trans-illuminator table but under natural lighting it was not possible to discern between marked and un-marked individuals. This study showed that high quality photography of marked specimens is possible under UV light using a 35-mm camera loaded with Kodak EPP 100+ Ektachrome film in combination with a UV-absorbing filter. It is estimated that one person could easily mark thousands of elvers in one hour and that about one pound of elvers (~2,500 individuals) can be marked with about $200 worth of calcein solution which can be re-used if necessary. In addition to using this mark for examination of short-term migration patterns, the potential exists for tracking marked elvers as they change hands in commercial marketing activities.

Study Number: LM-98-04

Title: Effect of water hardness on uptake of oxytetracycline and calcein marks in larval Atlantic salmon caudal fin tissue

Investigator: Jerre W. Mohler- Northeast Fishery Center-Lamar, PA (NEFC)

Background and Justification:

Fry stocking by the U.S. Fish and Wildlife Service has become an increasingly important part of the Atlantic salmon restoration program in the Northeastern U.S.. Allowing nature to select those which are fit for their environment is currently viewed as an important management tool to promote establishment of self-sustaining populations over their historic range. Paramount to the success of fry stocking in achieving management goals is the ability to assess the effectiveness of such a management strategy. Therefore, a need exists within Region 5 of the U.S. Fish and Wildlife Service for a technique of marking non-feeding Atlantic salmon fry (sac-fry) with a recognizable tag or mark capable of being detected in returning adult fish and other life stages. Since 1995, NEFC biologists have been inducing calcein marks in Atlantic salmon fry with promising results in application and detection of the mark. In 1997 *(Study L-97-01)*, results suggested a possible interaction between water hardness and other test variables in mark uptake for calcein. Due to chemical costs and wastewater handling concerns, efficient means of calcein-mark application must be discovered, therefore we initiated this study to examine the relationship between calcein uptake and water hardness. Calcein is not an FDA-approved chemical for use on fish and experimentation is necessary to contribute to the body of knowledge on use of this compound for purposes of gaining future FDA approval.

Study Objectives

We compared (1) effects of two water hardness and three marking chemicals on calcein mark readability at 30 days post-immersion in 4800 non-feeding ATS immersed for 48 hours at 250 mg/L concentrations and (2) 30-day mortality and growth between treatments.

Materials and Methods:

Immersion trials consisted of static baths of calcein, DCAF, and Oxytetracycline-343. Each treatment had 3 replicates in 6½-liter a acrylic hatching jars each containing 200 non-feeding ATS fry of Connecticut River domestic parental origin. Chemical immersions were prepared at concentrations of 250 mg/L. Immersion treatments were performed in both untreated NEFC spring water, NEFC water treated with a water softener, and untreated controls. Temperature of replicates was maintained at 12°C. At the end of 48-hour immersion, the immersion chemical was replaced with fresh flow-through water. As soon as possible post-immersion, 30 fish from each replicate were examined under UV microscopy and scored for mark readability as follows: 1=no mark; 2=dim, but detectable; 3=medium brilliance; 4=brilliant. A supply of live Artemia sp.along with a small amount of formulated salmon starter was introduced to each replicate daily with as much equity as possible. Calcein chemicals are not FDA-approved for fish use, therefore exposed fish will never be consumed or released into the wild but will be held captive at NEFC for future mark detection analysis.

Results

Four-day survival in all hard water (50 mg/L as $CaCo_3$) treatments was >97% while soft water (<5 mg/L as $CaCo_3$) treatments had nearly 100% mortality in chemical treatments and 57% mortality in controls. Mark determination showed that all fish immersed in DCAF and calcein (hard water treatments) had similar mark uptake with little variation in mark quality (no fish were scored as poorly marked). No mark was detected in oxytetracycline-immersed fish. Water hardness levels measured in soft-water treatments (<5 mg/L) were below tolerance limits of Atlantic salmon sac-fry as reflected by high mortality of treatment and controls. Therefore, no comparison of calcein mark uptake was made between hard and soft water treatments.

Study Number: LM-98-05

Title: Study of larval rearing density with hatchery-produced Atlantic sturgeon (Acipenser oxyrhincus).

Principal Investigator: Jerre W. Mohler; Northeast Fishery Center-Lamar, PA
Co-Invest/Cooperators: Kim King and Pat Farrell; Northeast Fishery Center-Lamar, PA

Background and Justification:

Commercial records of Atlantic sturgeon landings from the late 1800's to the present indicate a severe decline in the fishery (Taub, 1990). This problem has been addressed in the form of management plans for restoration of this species throughout its range by the Atlantic States Marine Fisheries Commission (ASMFC). The ASMFC culture and stocking group, which is comprised of all ASMFC states, the U.S. Fish and Wildlife Service, and the National Marine Fisheries Service, has made various recommendations in Special Report No. 22 titled: Recommendations Concerning the Culture and Stocking of Atlantic Sturgeon. including: "Basic cultural experiments should be undertaken at appropriate federal and state facilities to provide information on .....fry production techniques, rearing and holding densities...". Since 1993, NEFC has successfully spawned wild Hudson River ASN and produced from 12,000 to 160,000 fry annually but relationships between culture stocking density, rearing unit substrate area, rearing unit volume, and feed item density are unknown for early life stages of this species. It is important to discover these relationships for efficient hatchery operation and for obtaining greatest fry survival whether rearing fish for restoration stocking purposes or for commercial aquacuture.

Study Objectives

A total of 14,100 larval ASN will be used at four different stocking densities and two feed rates to compare effects on mortality and growth over a 20-day period. In addition, effect of increasing the area of available horizontal feeding substrate on growth and survival will be studied.

Materials and Methods

Larvae from Atlantic sturgeon eggs incubated at NEFC were used in the experiment. Prior to yolk sac absorption, larvae were pooled and randomly distributed into 60-L tanks at 200, 400, 600, 800, 1000, and 1200 fish per tank (3.70 - 22.2 fish/Liter) in triplicate. Additionally, one treatment contained increased feeding substrate area, one received additional daily feed ration, and one contained water at ½ the volume of a similar density. Water temperature were held at 16EC after and live brine shrimp Artemia sp. were introduced to all replicates via bellows pumps which were activated for 5 min. each ½ hour. Numbers of Artemia offered daily were quantified by doing random daily counts. Mortality was recorded daily. At the end of the 26-day experiment, biomass was determined for each replicate and 30 fish from each tank were individually measured for length/weight comparisons and to determine growth rate. Fish were then offered a formulated diet (Biokyowa C-400) and observed for the ability to convert from live to formulated feed.

Results

Survival was significantly lower (35%) in the treatment which contained additional grazing substrate area due to an infestation of Chilodinella sp., a parasitic protozoan. All other treatments had survival > 93%. Regression analysis showed Mean Specific Growth Rate (logarithmic growth per unit time) to be inversely proportional to fish density and curvilinear in form ranging from 6.3% in the high density to 10.9% in the low density treatment. In the first seven days post-study, only fish reared at the two lowest densities were able to convert to Biokyowa C-400 with little or no mortality. The minimum length and weight (±SD) for successful conversion to Biokyowa was observed to be 34.5 (3.0) mm and 0.18 (0.05) g, respectively. Information resulting from this study can be used with respect to potential stock restoration programs and for future commercial aquaculture.

Study Number: LM-98-06

Title: Comparison of growth and mortality in first-feeding Atlantic sturgeon fry when offered live Artemia sp., frozen Artemia, or a formulated diet

Principal Investigator: Jerre W. Mohler; Northeast Fishery Center-Lamar, PA
Co-Invest/Cooperators: Kim King and Patrick Farrell, Northeast Fishery Center-Lamar, PA

Introduction

Commercial records of Atlantic sturgeon (ASN) landings from the late 1800's to the present indicate a severe decline in the fishery. This problem has been addressed in the form of management plans for restoration of this species throughout its range by the Atlantic States Marine Fisheries Commission (ASMFC). The ASMFC culture and stocking group, which is comprised of all ASMFC states, the U.S. Fish and Wildlife Service, and the National Marine Fisheries Service, has made various recommendations in Special Report No. 22 titled: Recommendations Concerning the Culture and Stocking of Atlantic Sturgeon. including: "Basic cultural experiments should be undertaken at appropriate federal and state facilities to provide information on .....nutritional requirements and feeding techniques...". Earlier diet trials with hatchery produced ASN fry at the Northeast Fishery Center (NEFC)- Lamar showed that fish were larger and had greater survival on live brine shrimp (Artemia sp.) or live Artemia supplemented with a formulated diet as opposed to all other formulated diets offered. Like most species of fish, maintenance of sturgeon fry is labor intensive. Production and feeding of Artemia adds a great deal of additional labor to the sturgeon rearing process. Therefore it would be desirable to produce and freeze Artemia at times when the need for labor is not as great. This experiment will determine whether first-feeding ASN fry will accept, survive, and grow on frozen Artemia at a similar rate to those fed live Artemia.

Study Objectives

About 3600 first-feeding Atlantic sturgeon fry were fed either live or frozen Artemia nauplii, or Biokywa B-250 formulated diet for the first 26 days of feeding to compare mortality and growth between treatments at the Northeast Fishery Center- Lamar, PA.

Materials and Methods:

Larvae from Atlantic sturgeon eggs incubated at NEFC were used in the experiment. Prior to yolk-sac absorption, larvae were pooled, randomly distributed into 60-L tanks, and assigned one of the three diet treatments in triplicate. Four hundred fry were placed in each tank. Flow rate was 2-3 liters/min. and temperature was 16 °C. Prior to introduction of feed, average fry length/weight was determined. Bioykowa tanks were equipped with automatic feeders to dispense feed over 24 hours and live Artemia culture was fed using bellows pumps which were automatically activated to supply feed for 5 min. each ½ hour. Frozen Artemia tanks each received 3 cubes of feed daily (AM, noon, and PM). Numbers of Artemia nauplii fed were determined by performing sample counts on brine shrimp solutions prior to freezing and on live cultures. Sturgeon mortality was recorded daily. At the end of 26 days, biomass was determined for each replicate using the wet weight method and average lengths were obtained.

Results

Fish survived poorly in the Biokyowa treatment with mean survival of 13% vs. live or frozen Artemia treatments at >93% over the 26-day study. Even though survival was similar between sturgeon in live and frozen Artemia treatments, fish fed frozen nauplii were shorter (22.5 vs. 34.5 mm total length) and weighed less (0.05 vs. 0.18 grams). Sample counts showed that frozen and live Artemia treatments received similar numbers of nauplii (1.30×10^7 vs. 1.38×10^7), respectively over the study period, but frozen Artemia tanks were fed three times daily as opposed to live Artemia tanks which were fed every ½ hour for 5 minutes duration. Our study showed that frozen Artemia is a promising first feed for sturgeon and may rival growth obtained with live Artemia if feeding frequency is increased.

Study Number: LM-98-07

Title: Study of rearing density with fingerling-size hatchery-produced Atlantic sturgeon *(Acipenser oxyrhincus)*.

Principal Investigator: Jerre W. Mohler, Northeast Fishery Center-Lamar, PA
Co-Invest/Cooperators: Kim King and Pat Farrell, Northeast Fishery Center-Lamar, PA

Background and Justification

Commercial records of Atlantic sturgeon (ASN) landings from the late 1800's to the present indicate a severe decline in the fishery. This problem has been addressed in the form of management plans for restoration of this species throughout its range by the Atlantic States Marine Fisheries Commission (ASMFC). The ASMFC culture and stocking, which is comprised of all ASMFC states, the U.S. Fish and Wildlife Service, and the National Marine Fisheries Service, has made various recommendations in Special Report No. 22 titled: Recommendations Concerning the Culture and Stocking of Atlantic Sturgeon. Recommendation 1.4 states: "Basic cultural experiments should be undertaken at appropriate federal and state facilities to provide information on ..... production techniques, rearing and holding densities...". Since 1993, NEFC has successfully spawned wild Hudson River ASN and produced from 12,000 to 160,000 fry annually but relationships between culture stocking density, rearing unit substrate area, rearing unit volume, and feed item density are unknown for early life stages of this species. It is important to discover these relationships for efficient hatchery operation and for obtaining greatest fry survival whether rearing fish for restoration stocking purposes or for commercial aquacuture..

Study Objectives

About 3,000 fingerling ASN (approx. 0.4 grams each) will be used at five different stocking densities using a 3% feed rate to compare effects on mortality and growth over a 30-day period. In addition, effect of increasing the area of available horizontal feeding substrate on growth and survival will be studied.

Materials and Methods

Fingerlings produced from Atlantic sturgeon eggs incubated at NEFC will be used in the experiment. Prior to study, fish will be pooled and randomly selected for inclusion into 60- L circular tanks at densities ranging from 25-175 fish per tank (0.37-2.22 g/L) respectively. Zeigler sturgeon diet (#2 or #3 crumbles) will be introduced at 3% body weight daily via automatic feeders. Light regime will be natural photo-period. Flows will be adjusted to 4 L/min. Temperature will be set for 16°C. In addition, one treatment will contain an additional horizontal platform to increase available feeding and resting area.

Results

At the end of 30 days, percent mortality (±SE) ranged from 4.7 (0.6) to 13.0 (0.0) and was lowest in the treatment containing additional substrate. Fish from all treatments were similar in weight and length after 30 days. Feed conversion (weight of feed offered/wet weight gain) (±SE) ranged from 0.44 (0.02) in the low density treatment to 0.56 (0.03) in the treatment which contained additional substrate and was significantly different between the two. Results showed that in our study densities were not high enough to adversely affect growth (the high density treatment had a final mean tank biomass of 6 grams/liter). Atlantic sturgeon are primarily benthic feeders and fish were observed to vigorously compete for food when it sank to the bottom of the tank. Fish in tanks containing additional substrate did not show a growth benefit but showed less mortality indicating that the additional area may have provided additional resting areas thus reducing individual stress. Random observations showed that as many as 10% of fish in additional substrate tanks utilized the platform.

Study Number: LM-98-08

Title: LC50 determination for three therapeutic chemicals on Atlantic sturgeon (Acipenser oxyrhincus) fingerlings.

Principal Investigator: Kim King; Northeast Fishery Center-Lamar, PA

Background and Justification:

Therapeutic chemicals are routinely used to treat fish diseases in aquacultural practice if the presence of parasites and or disease in fish is diagnosed. Current interest in development of guidelines for culture and restoration of Atlantic sturgeon (ASN) has prompted federal government and state agencies to place more emphasis on rearing techniques for this species. Increased fish production is often accompanied by an increase in fish diseases and requires use of therapeutic chemicals. Although use of these chemicals has many advantages, they also pose problems such as toxicity to the fish they are used on. The toxicity of a chemical should be known for the particular species to be treated but little is known about the sensitivity of ASN to chemicals and drugs used in aquacultural practice. This experiment will examine mortality of ASN fingerlings exposed to various levels of three chemicals commonly used in fish culture to prevent or treat disease: formalin, chloramine-T, and sodium chloride. A standard measurement of toxicity (96-hour LC50 values) will be determined. This is defined as the chemical concentration which results in 50% mortality of test organisms over 96 hours. Results of our study will provide fishery managers with guidelines for use of these chemicals as theraputic agents on ASN.

Study Objectives

We will (1) determine the 96-hour LC50 values for Atlantic sturgeon fingerlings exposed to three chemical agents, formalin, chloramine-T, and sodium chloride and (2) determine whether delayed effects occur after 96-hour exposure on 1998 year class ASN fingerlings at the Northeast Fishery Center-Lamar, PA

Materials and Methods

All bioassays were performed using 1998 year class ASN fingerlings (approximately 1.0 gram) produced from eggs incubated at the Northeast Fishery Center, Lamar PA. Fish were maintained according to the American Society for Testing and Materials (ASTM) standard procedures for handling fish for toxicity studies. Acute toxicity tests were conducted according to procedures prescribed by the ASTM and the Committee on Methods for Toxicity Tests with Aquatic Organisms. In each test, 126 fish were exposed for 96 hours to selected concentrations of test material in glass jars containing 19 L of oxygen-saturated dilution water. Each test consisted of a control and five concentrations (21 fish per treatment, divided between three replicates). Exposure concentrations were determined from range-finding tests consisting of at least one 48-hour exposure of test fish to five chemical concentrations and fresh water (controls). Fish mortality was recorded at 3, 6, 12, 24, 48, 72, and 96 hours after test initiation, and dead fish were removed daily. At the end of each test, survivors were placed in fresh water for 2-8 days to determine delayed effects. Mortality data was analyzed by the Probit method to calculate LC50 values and 95% confidence intervals.

Results

The 96-hour LC50's (concentrations calculated to produce 50% mortality in a population) were: formalin (31.001Fl/L), chloramine-T (7.66 mg/L), and sodium chloride (9.735 g/L). These values are useful measurements of relative acute lethal toxicities of these chemicals to Atlantic sturgeon fingerlings under our specified conditions. Our study showed that Atlantic sturgeon fingerlings were safely exposed to 24-hour static bath concentrations at the following levels: formalin (54.0 - 90.0 Fl/L), chloramine-T (6.7 - 11.2 mg/L), and sodium chloride (7.13 - 11.88 g/L).

Study Number: LM-98-09

Title: **Effect of density on mortality of green and eyed Atlantic salmon eggs and size of alevins in vertically-stacked incubator trays at White River National Fish Hatchery (Phase II)**

Principal Investigator: Jerre W. Mohler-Northeast Fishery Center-Lamar, PA (NEFC)
Co-investigators: Ken Gillette and Bruce Jensen-WRNFH; Michael Millard-NEFC

Background and Justification:

In 1997, Phase I of this investigation was performed with eggs of domestic Connecticut River Atlantic salmon (ATS) at White River National Fish Hatchery (WRNFH). A large proportion of eggs produced in Region 5 salmon hatcheries are incubated there due to favorable water temperatures and facilities. In some years, egg production nearly exceeds available incubation space, therefore it is necessary to optimize use of existing incubation facilities. This can be accomplished by incubating more eggs per tray but effects of elevated egg densities on egg mortality and alevin size are not known for this species. In 1997, we tested green eggs at 8,000 - 12,000 per tray (eyed-eggs at 6,500 - 10,500 per tray) and found that percent mortality, blue sac, and deformed fry were not different between treatments. Furthermore, alevins were larger at the elevated densities than those incubated at normal production levels. This study will have 3 components: (1) testing highest Phase I density in a production mode (full stack), (2) Testing a density higher than Phase one levels (14,000 green eggs / 12,000 eyed eggs per tray) and, (3) stocking kelt eggs from North Attleboro NFH at three densities equivalent in volume to those used for domestic eggs in the Phase I study (L-97-04).

Study Objectives

We will compare effects of two egg densities (at a production level) on mortality and alevin size using 660,000 green and 570,000 eyed Atlantic salmon eggs at WRNFH during the 1998/99 incubation period. Additionally, we will compare effects of egg density on mortality and alevin size with 14,000 green/12,000 eyed per tray. Finally we will compare effects of three egg densities on mortality and alevin size of an undetermined number of kelt eggs. Through data analysis, we will recommend a maximum egg density per tray for future incubation of ATS eggs at WRNFH.

Materials and Methods

The study was performed at WRNFH during the 1998/99 incubation year. **Experiment #1.**-660,000 eggs from domestic ATS were composited in a net suspended in hatchery water after iodophor disinfection. Eggs were enumerated and placed into four full stacks of incubator trays. Two stacks contained 10,000 and two contained 12,000 eggs/tray. **Experiment #2.**- 316,000 fertilized eggs were composited and placed into trays at three densities (10,000 - 12,000 - 14,000). Each treatment had two replicates at each of three tray levels (high, medium, low). The balance of trays throughout the stacks were stocked at densities normally maintained at WRNFH. **Experiment #3.**- We composited 20,600 mls of kelt eggs from N. Attleboro NFH and established trays at three densities: 910, 1140, and 1380 mls per tray (based on egg volumes tested in Phase I experiment, study number L-97-04. The balance of trays throughout experimental stacks contained kelt eggs at densities normally maintained at White River NFH. Once eggs have reached the eyed stage, mortality will be compared between treatments and eggs will be composited within their experimental densities put into Heath trays as before but at reduced densities commonly used at WRNFH. Eggs will be allowed to hatch and held in Heath trays until yolk sac absorption. The study will conclude after performing final larval inventories.

Results

The study is on-going with results expected in the Spring of 1999.

OTHER BIOLOGICAL INVESTIGATIONS PERFORMED:

LM98A **Tank spawning of America shad using time-released hormone implants.-** The Service and partners in the Susquehanna River Anadromous Fish Restoration Committee have been involved in the restoration of American shad (AMS) to the Susquehanna River for a number of years. Traditionally the egg source for AMS culture has involved lethal collection at a considerable cost. For the past two years, tank spawning of AMS injected with time-release dose implants of Luteinizing Hormone-Releasing Hormone analogue (LHRHa) have been conducted at the Manning SFH in Maryland and the Waldoboro Shad Hatchery in Maine. The Northeast Fishery Center (NEFC) is undertaking a cooperative effort to conduct and develop tank spawning technology with the goal of establishing a spawning sub-population of American shad imprinted to return and use the West Branch of the Susquehanna River. Annual production requirements of 10 million fertilized shad eggs for the Pennsylvania Fish and Boat Commission, Van Dyke Shad Hatchery and two million marked fry to be stocked by the NEFC in the West Branch of the Susquehanna River have been set for the next five year period.

Work conducted by NEFC in FY 98 included: Construction of a 13,500-gallon recirculating tank spawning unit in the Intensive Culture Building; assembly of an incubation and 4,000-gallon fry rearing system in the Hatchery Building, and pilot production efforts. Major spawning system components include: LHRHa (luteinizing hormone - releasing hormone analogue) implants, three circular and one cross flow rectangular brood tanks, four egg collection tanks, sump, duplex controlled pumps, propane heaters, shell-in-tube heat exchanger, degassing column, UV light disinfection unit, oxygen generator and injector, and an alarm system. The incubation and fry rearing systems consist of : Tempered water supply, water softener, forty 6.5-liter incubation jars, eight 500-gallon culture tanks, and an alarm system. Production efforts were impaired by high water conditions at the Conowingo fish lift on the lower Susquehanna River, which precluded collection of potential brood during the most productive portion of the run, early to middle. In all, 221 females and 290 male AMS were captured and transported to the NEFC under contract from May 21 to June 4, 1998. A total of 3.2 million eggs were collected. Of that number, 1.8 were shipped to the Van Dyke Hatchery and the remainder were incubated at the NEFC. Fry were fed Artemia sp. starting day 3 and were reared to day 15 to enable four bath oxytetracycline marking applications at NEFC. A total of 50,000 thousand marked American shad were released into the West Branch of the Susquehanna by NEFC at McElhatten, Pennsylvania.

LM98B **Stream-side spawning of a female Atlantic sturgeon using oviduct puncture technique and transport of fertilized eggs.-** Northeast Fishery Center personnel in cooperation with the New York Department of Environmental Conservation spent over 2 weeks on the Hudson River near Esopus Island during sturgeon spawning season for purposes of capturing and spawning ripe broodstock. A total of 84 different males and three females were captured, measured, and tagged. Of the three females captured, one was spent, one was in a hard-roe condition, and one was running with ripe eggs. Since the ripe female was actively shedding eggs, it was transported via boat to the Norrie Marina and placed into NEFC's sturgeon transport unit (STU). The fish was turned onto its back and supported via ropes near the STU tank water surface where spawning procedures commenced. A small scalpel was inserted into the vent and a puncture was made in the left oviduct which allowed about 2 quarts of eggs to be extruded into a 5-gallon plastic bucket. Milt from 5 males was used to fertilize the eggs which were then mixed with river silt to prevent clumping. Eggs were transported in a 5-gallon bucket equipped with oxygenation via an air stone during the 6-hour journey back to Lamar, PA. Egg cell division was quite advanced by the time we arrived at Lamar to place eggs in incubation jars. Thirty-six-hour post-fertilization egg samples showed about 80% hatch rate producing nearly 124,000 fry. First-feeding fry were involved in early rearing experiments where valuable information concerning density and feeding techniques were discovered. The first successful pond-culture of feeding fry was also performed producing about 5,000 healthy fingerlings. Nearly 5,700 fingerlings were given to a total of seven different cooperators and

researchers for experimentation to increase knowledge of disease susceptibility, culture, and environmental requirements of this species. In-house Toxicity studies using three commonly-used hatchery chemicals were also performed with the fingerlings. In addition, at least 100 individuals will be maintained at NEFC as future broodstock along with the other four year-classes present.

LM98C Pond culture of Atlantic sturgeon (feeding fry).- After about 3 weeks of tank culture, supplies of live feed (brine shrimp) were depleted, therefore nearly 70,000 fry were moved outdoors to a 1/3 acre pond which had been fertilized 12 days prior. The pond was operated on a flow-through mode and supplemented with 1.5 Kg of Biodiet formulated feed daily. Water temperature was about 25.5°C, pH was about 9.0, and dissolved oxygen was measured at 6.7mg/L. After 28 days, the pond was drawn down and harvested resulting in the recovery of nearly 5,000 healthy fingerling Atlantic sturgeon which were shipped to other researchers for various studies. To our knowledge, this was the first successful pond culture of early-life stage Atlantic sturgeon.

LM98D Fish Health Inspection Services provided to National Fish Hatcheries in Region 5.-In compliance with the Service Fish Health Policy and Implementation Guidelines, Great Lakes Fish Disease Control Policy and Model Program, and the New England Salmonid Health Guidelines, the Lamar Fish Health Unit has conducted annual or semiannual fish health inspections at Service fish rearing facilities. These statistically based fish health examinations are essential to stop the spread of fish diseases through fish transport and are necessary to enable facilities to legally release or transport fish. Those facilities working with limited valuable and imperiled stocks, such as Atlantic salmon, are able to modify the annual inspection requirements through mortality monitoring, where hatchery staff are trained by fish health biologists to sample the recent mortalities and send the samples to the fish health diagnostic laboratory at Lamar. This procedure, though more work intensive at the lab, provides a better, year-round surveillance of the health status of the fish stocks as well as reduces the number of valuable fish needed to be sacrificed.

LM98E Participation in the National Wild Fish Health Survey.- Under the leadership of Service Regional Fish Health Centers, and in cooperation with many stakeholders, this project was launched in 1997 in order to determine the geographic distribution of certain pathogens in the United States. The Survey is incorporating standardized diagnostic techniques and data management methods to ensure national comparability; identifying target pathogens, fish species, and habitats; and developing a systematic watershed approach. In fiscal year 1998, the Lamar Fish Health Unit sampled 1,815 fish representing 19 species. Over 30 sites have been sampled, including sites along the Hudson, Susquehanna, and Connecticut Rivers, three of the rivers designated as American Heritage Rivers. To date, National Wild Fish Health Survey work has been conducted in ten of the thirteen states in Region 5.

LM98F Cooperative work on a newly found virus in Atlantic salmon.-As part of the mortality monitoring program at the North Attleboro NFH, a condition of many tumors in and along the swimbladders in Pleasant River origin Atlantic salmon was investigated. Researchers at the Cornell University Center for Veterinary Medicine working on the problem, made the first identification of a virus believed responsible for cancerous tumors in Atlantic salmon in the New England area. The next step in studying the virus is investigating virus transmission in a cooperative effort with Cornell Univ. and the USGS-BRD national Fish Health Research Lab. It is also planned to examine various captive and wild populations of Atlantic salmon.

LM98G Participation in the Service National INAD Program.- The Lamar Fish Health Unit participates in the Investigational New Animal Drug (INAD) program by providing study monitors to all the participating field stations, forwarding all drug shipment and use records, and serving as Regional INAD Coordinator to the National INAD Office.

LM98H Participation in Maine Fish Health Advisory Board concerning ISAv issues - The Maine Fish Health Advisory Board serves as a scientific advisory board to the state Commissioners for the Dept. of Marine Resources, and Inland Fish and Wildlife on fish health issues. A Service representative from the Fish Health Unit serves on the board. The group has no regulatory power, but has been heavily involved in the Infectious Salmon Anemia virus (ISAv) and issues related to private aquaculture and wild resources. Because of its geographic proximity to the ISA infected Canadian sites, the Board is accepting the probability that ISA will show up in Maine soon. An intensive program of monitoring for ISA in the Maine salmon industry was initiated during the summer of 1998. So far, the virus has not been detcted from any of the private aquaculture sites tested this year in Maine. Federal and state participants in the **Atlantic Salmon Restoration Program** also implemented practices which will minimize affects of ISA on migrating populations. The Lamar Fish Health Unit initiated additional laboratory procedures to screen for ISA in samples received from all sea run Atlantic salmon mortality and reproductive fluid samples. The virus has not yet been detected in any wild salmon tested.

LM98I Pro-active Fish Health Management at Atlantic salmon facilities - In cooperation with several facilities in Region 5 at risk for disease epizootics, the Fish Health Unit coordinates and administers a variety of programs designed to monitor for and prevent such incidents. The detection techniques include non-lethal sampling of water, salmonid mucus, and reproductive fluids for detection of bacterial and viral diseases. These techniques allow for identification of pathogens before they can cause mortality or be spread to other facilities through gametes. They allow adequate time to treat or manage the pathogens in appropriate ways to minimize or eliminate their impact on Atlantic salmon restoration efforts. Preventative management strategeies are also administered. Vaccination against furunculosis has been employed at production facilities at risk for contracting the disease. The Atlantic salmon Sea-run Protocol, which administers a combined antibiotic/vaccine injection to each fish upon arrival to sea-run broodstock facilities in lower New England, has been a very successful preventative of bacterial epizootics during spawning season. All pro-active protocols involve partnerships with several state and Service salmon facilities, the Fish Health Unit, and USGS-BRD.